Current Biology 24, 646–651, March 17, 2014 ©2014 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2014.02.005

Report

Slow Checkpoint Activation Kinetics as a Safety Device in Anaphase

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Summary

Chromosome attachment to the mitotic spindle in early mitosis is guarded by an Aurora B kinase-dependent error correction mechanism [1, 2] and by the spindle assembly checkpoint (SAC), which delays cell-cycle progression in response to errors in chromosome attachment [3, 4]. The abrupt loss of sister chromatid cohesion at anaphase creates a type of chromosome attachment that in early mitosis would be recognized as erroneous, would elicit Aurora B-dependent destabilization of kinetochore-microtubule attachment, and would activate the checkpoint [5, 6]. However, in anaphase, none of these responses occurs, which is vital to ensure progression through anaphase and faithful chromosome segregation. The difference has been attributed to the drop in CDK1/cyclin B activity that accompanies anaphase and causes Aurora B translocation away from centromeres [7–12] and to the inactivation of the checkpoint by the time of anaphase [10, 11, 13, 14]. Here, we show that checkpoint inactivation may not be crucial because checkpoint activation by anaphase chromosomes is too slow to take effect on the timescale during which anaphase is executed. In addition, we observe that checkpoint activation can still occur for a considerable time after the anaphase-promoting complex/cyclosome (APC/C) becomes active, raising the question whether the checkpoint is indeed completely inactivated by the time of anaphase under physiologic conditions.

Results and Discussion

Relocalization of Checkpoint Proteins to Kinetochores in Anaphase Does Not Prevent Degradation of APC/C Substrates

In several organisms, artificial maintenance of high cyclin B levels in anaphase results in recruitment of spindle assembly checkpoint proteins to kinetochores when sister chromatids split [7, 10, 12]. Yet, whether this kinetochore recruitment indeed creates a signal sufficient to inhibit the anaphase-promoting complex/cyclosome (APC/C) has remained unclear, and the observation that APC/C substrates are degraded in this situation suggests that it may not [15]. To systematically test this, we expressed physiologic amounts of nondegradable cyclin B (ΔN-Cdc13) in fission yeast (*S. pombe*) mitosis (Figure S1A available online). As expected [16], sister chromatids separated in anaphase but cells maintained short metaphase-like spindles (pseudometaphase; Figure S1B). The Aurora B kinase (*S.p.* Ark1) was retained on centromeres (Figure 1A), and the Polo kinase Plo1 was retained on spindle pole bodies (Figures S1B and S1C), indicating that CDK1 activity remained high [17, 18]. As had been observed in other cell types [7, 12, 15], kinetochore attachment became unstable, and centromeres frequently detached from the spindle poles (Figures S1B and S1D). Consistent with the destabilization of kinetochore attachment, the checkpoint proteins Mad1, Mad2, Mad3, and Bub3 localized to kinetochores of pseudometaphase cells (Figures 1A and 1B). Rebinding of Mad3 was almost immediate with anaphase onset and occurred even before detachment of kinetochores from the spindle poles was observed (Figures 1B, 1C, S1D, and S1F). However, the accumulation of checkpoint proteins did not appear to inhibit the APC/C, since degradation of the APC/C substrates cyclin B (*S.p.* Cdc13; Figure 1D) and securin (Figures 1D and 1E) continued unhindered. This suggested that the checkpoint does not detectably block APC/C activity when cyclin B is maintained, although checkpoint proteins enrich at kinetochores.

The MCC Forms in Anaphase when Nondegradable Cyclin B Is Present

To determine at which step checkpoint signaling may be blocked, we analyzed the formation of the mitotic checkpoint complex (MCC). The MCC is the ultimate inhibitor formed by the spindle assembly checkpoint [3] and consists of the APC/C activator Cdc20 (*S.p.* Slp1) and the checkpoint proteins Mad2 and Mad3. We synchronized cells expressing nondegradable cyclin B at the G2/M transition and immunoprecipitated the APC/C subunit Lid1 (Apc4) from cells in metaphase (20 min after release) or in pseudometaphase (28 min after release) (Figures 2A, S2A, and S2B). In pseudometaphase, but not in metaphase, Mad2 was clearly associated with APC/C and Slp1, indicative of MCC formation. In contrast, in the absence of nondegradable cyclin B, Mad2 did not accumulate on the APC/C during anaphase. The amount of MCC formed in pseudometaphase seemed substantial, as we coimmunoprecipitated less Slp1 and Mad2 from a culture where the checkpoint was engaged by treatment with the microtubule-destabilizing drug MBC (Figures S2C and S2D). Hence, MCC formation does take place when cyclin B levels remain high, but seems unable to prevent degradation of APC/C substrates.

Separase Activity Is Insufficient to Inactivate the Spindle Assembly Checkpoint

Separase overexpression overrides a mitotic checkpoint arrest in budding yeast [10, 13]. Because anaphase coincides with separase activation, we reasoned that separase might block a late step in checkpoint activity. To test this hypothesis, we conditionally overexpressed separase. In this situation, separase is not reliably inhibited by securin, and sister chromatids split almost instantaneously when cells enter mitosis (Figure 2B). Cohesin mutations that induce a similar precocious loss of cohesion cause a checkpoint-dependent delay in mitosis [19, 20]. If separase activity was sufficient to block checkpoint signaling, separase-mediated cohesion loss should not delay cells in mitosis [10]. However, we

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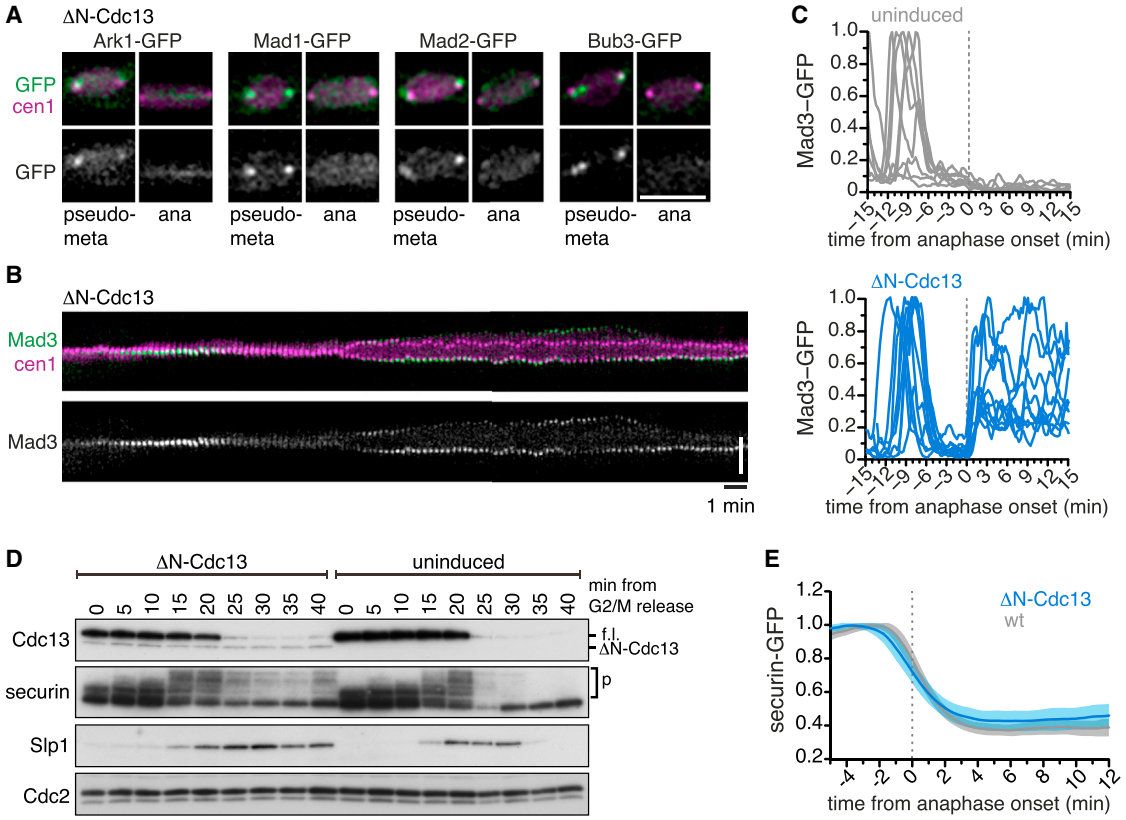

Figure 1. Securin and Cyclin B Are Degraded in Pseudometaphase Despite Fast Reaccumulation of Checkpoint Proteins at Kinetochores

(A and B) Expression of nondegradable cyclin B (ΔN-Cdc13) was induced in cells expressing the indicated proteins fused to GFP and a marker for the centromere of chromosome I (cen1). Mitotic progression was followed by live-cell imaging. Representative nuclei of cells 2 min after sister chromatid separation are shown in (A) (scale bar, 4 μm). Only cells exhibiting a pseudometaphase phenotype show localization of checkpoint components after anaphase onset. A representative kymograph of a cell expressing *mad3*$^{+}$*-GFP* and exhibiting a pseudometaphase phenotype is shown in (B) (vertical scale bar, 5 μm; see Figure S1E for comparison to the wild-type). Localized Mad3-GFP signal decreases in metaphase but reaccumulates quickly after anaphase when ΔN-Cdc13 is present (timing quantified in Figure S1F).

(C) Quantification of the experiment shown in (B). The maximal cellular Mad3-GFP signal was determined in individual cells, either without induction of ΔN-Cdc13 (gray, n = 8) or with induction of ΔN-Cdc13 and pseudometaphase phenotype (blue, n = 11). Individual time courses were aligned to the point of sister chromatid separation.

(D) *Cdc25-22* cells with or without induction of ΔN-Cdc13 were synchronized at the G2/M transition. Samples taken at the indicated time points were analyzed by immunoblotting using anti-Cdc13, anti-Cut2 (securin; antibody characterization in Figure S1G), anti-Slp1 and anti-Cdc2 (Cdk1, loading control) antibodies. p indicates mitotic phosphorylation of securin, f.l. marks the endogenous cyclin B, and ΔN-Cdc13 the shorter nondegradable version of Cdc13. Slp1 (*S.p.* ortholog of Cdc20) is stabilized as a result of the expression of ΔN-Cdc13. Endogenous Cdc13 accumulates in a *cdc25-22* arrest, so that in this experiment there is less ΔN-Cdc13 than f.l. Cdc13. See Figure S1A for a comparison in unsynchronized cells. Quantification of the cell-cycle stages is shown in Figures S1H and S1I.

(E) The abundance of securin-GFP was followed by live-cell imaging in wild-type cells (gray, n = 31) or in cells displaying a pseudometaphase phenotype after induction of ΔN-Cdc13 (blue, n = 22). The cen1 marker was used to determine the onset of anaphase and individual time courses were aligned to this point. Shown is the average (line) ±SD (filled area).

See also Figure S1.

observed a mitotic delay in separase-overexpressing cells, which furthermore was checkpoint dependent as deletion of the checkpoint gene *mad2*$^{+}$ abolished the delay (Figures 2B and 2C). Hence, we conclude that separase activity does not inactivate the spindle assembly checkpoint in fission yeast.

Checkpoint Reactivation in Anaphase Is Slow Relative to the Timing of Securin Degradation

The MCC formation in cells undergoing anaphase in the presence of nondegradable cyclin B (Figure 2A) suggested that checkpoint reactivation is not completely blocked, but that either the APC/C had become refractory to inhibition or that checkpoint reactivation is too slow to manifest in a block of securin degradation, i.e., the block in APC/C activity would only occur at a time when securin is completely degraded. To test the latter idea, we gave cells more time between the onset of anaphase and the completion of securin degradation. We overexpressed securin to about eight times its wild-type level (Figure S3A). This increased the total time of securin degradation from 5.5 min to 8.2 min (Figures 1E and 3A). However, the time between anaphase and complete securin degradation was still similar to wild-type cells (~3.7 min; Figures S3B and S3D). We therefore shifted the time of anaphase by co-overexpressing separase. As a result, anaphase occurred soon after the start of securin degradation and the time between the onset of anaphase and complete securin degradation was prolonged (7.6 min on average; Figures S3B and S3D). Interestingly, if we additionally stabilized cyclin B, securin degradation was halted before complete degradation (Figures 3A–3C). This depended on checkpoint

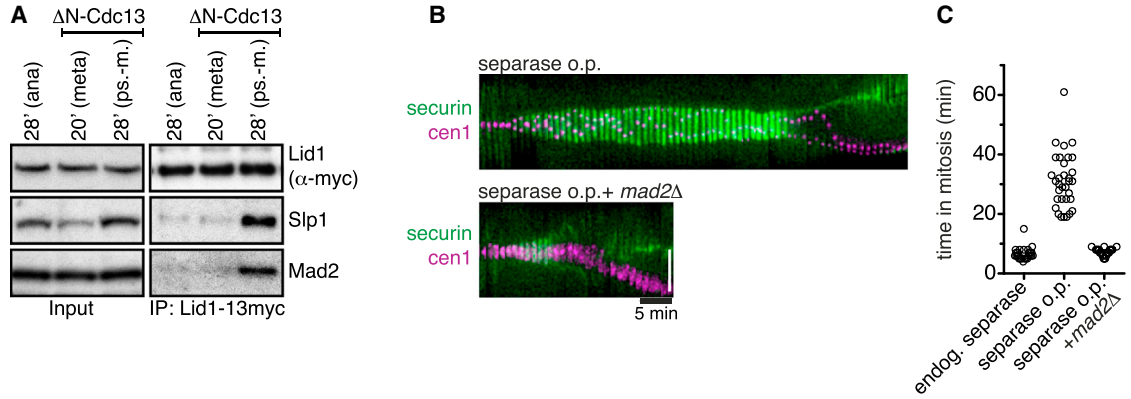

Figure 2. Expression of Nondegradable Cyclin B Results in Formation of the Mitotic Checkpoint Complex in Pseudometaphase

(A) *Cdc25-22* cells with or without induction of ΔN-Cdc13 were synchronized at the G2/M transition. Cells were harvested at the indicated time points after release, and the APC/C subunit Lid1 was immunoprecipitated and analyzed for coimmunoprecipitation of the MCC components Slp1 and Mad2 by immunoblotting. Quantification of the cell-cycle stages is shown in Figures S2A and S2B.

(B) Overexpression of separase was induced in cells carrying securin-GFP and a centromeric marker for chromosome I (cen1) in the presence or absence of the checkpoint protein Mad2. Mitotic progression was followed by live-cell imaging. Representative kymographs are shown (vertical scale bar, 5 μm).

(C) Quantification of the experiment in (B). The time in mitosis was determined by the presence of spindle-associated securin-GFP.

See also Figure S2.

activity, since deletion of the checkpoint gene *mad2*$^{+}$ allowed securin degradation to run to completion (Figures 3A–3C). Hence, the checkpoint indeed reactivates at anaphase if cyclin B is stabilized, but is slow in inhibiting the APC/C: it took between 3.5 min and 16 min (average 6.5 min) from anaphase to the stabilization of securin (Figure 3D). In cells solely expressing nondegradable cyclin B, which we had monitored earlier (Figure 1E), the time from anaphase to completion of securin degradation was only 1.5 min to 4.8 min (Figure S3D). Hence, securin degradation was completed or almost completed by the time the APC/C became inhibited, which explains our failure to observe securin stabilization in this background.

Since the time from anaphase onset to checkpoint protein recruitment was short (Figures 1B and 1C), this indicated that the time from checkpoint protein recruitment to APC/C inhibition is long. To test this directly, we monitored Mad2-mCherry signals in securin- and separase-overexpressing cells and related their kinetochore reoccurrence to the stabilization of securin (Figures 3E and 3F). As we had seen before, Mad2 enriched at kinetochores very soon after anaphase (0 to 60 s with an average of 30 s; see Figure 3E for an example), whereas it took 2.7 to 6 min (average 4.3 min) from the first enrichment of Mad2 until securin degradation was halted (Figure 3F). Hence, if anaphase occurs in the presence of nondegradable cyclin B, checkpoint protein rerecruitment to kinetochores is fast but APC/C inhibition is slow.

Checkpoint Reactivation Can Occur Once Cyclin B Degradation Has Started

Our results suggested that—although stabilization of cyclin B levels allows reactivation of the checkpoint in anaphase—reactivation is too slow to take effect before securin is degraded. It had previously been proposed that the checkpoint is completely inactivated by the time of anaphase [6, 14]. We therefore asked whether checkpoint activation became impossible or at least more inefficient when we allowed cyclin B levels to drop. To study this systematically, we turned to cells containing the kinesin-5 mutation *cut7-446* [21] and slightly impaired kinesin function by incubating at semipermissive temperature. We reasoned that this would cause spindle instability and frequent destabilization of chromosome attachments. Indeed, in some cells, we observed securin stabilization after the start of securin degradation (Figure 4). This stabilization appeared to be related to checkpoint signaling, because in the minutes preceding stabilization, strong Mad2 signals were observed, whereas in the minutes preceding securin degradation (either initially or when restarting), Mad2 signals were low (Figure 4C). This corroborates observations by the Pines and Gerlich groups, who showed that the checkpoint can still be activated after the APC/C has become active [22, 23]. Interestingly, the time between observing Mad2 signals and stabilization of securin levels was between 3 and 7 min (average 5.2 min), which is similar to the timing that we observed in cells with stabilized cyclin B (between 2.7 and 6 min; Figure 3F). When we plotted these values relative to the time that had elapsed since securin degradation started (Figure 4D), there was no obvious prolongation in the time needed for APC/C inhibition. This suggests that cyclin B degradation does not drastically alter the kinetics of checkpoint signaling, at least for about 2 min after the APC/C has become active. Because it takes around 2 min from the onset of securin degradation to anaphase in wild-type cells (Figure 1E), this raises the possibility that checkpoint signaling is still operational at anaphase. It should be noted that there is copious evidence that the checkpoint is inactivated at some point during mitotic exit, either through degradation of checkpoint proteins [14, 24–26] or through loss of CDK1-dependent phosphorylations [27–30]. However, when with respect to anaphase these mechanisms inactivate the checkpoint is largely unclear. Work from the Petronczki group indicates that recruitment of the checkpoint proteins Mad1 and Mad2 to kinetochores may be impaired by the time of anaphase in human cells [11].

Slow Checkpoint Activation May Protect Anaphase While Cyclin B Levels Are Still High

Our data suggest that the checkpoint remains operational at anaphase. Yet, in an unperturbed anaphase, rerecruitment of

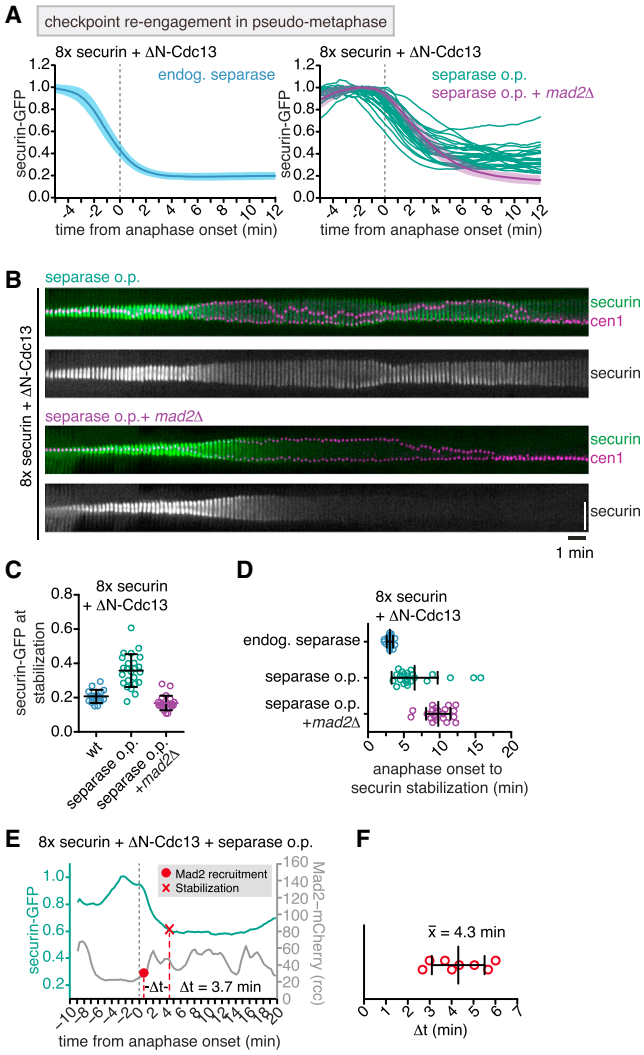

Figure 3. Checkpoint Re-engagement in Pseudometaphase Can Be Observed when the Time Period of Securin Degradation Is Prolonged

(A) Degradation kinetics of securin-GFP were assayed as in Figure 1E. In addition to nondegradable cyclin B (ΔN-Cdc13), cells overexpressed securin-GFP to about 8-fold (Figure S3A) (left, blue; n = 17), overexpressed securin-GFP and separase (right, green; n = 24), or overexpressed securin-GFP and separase and had *mad2* deleted (right, aubergine; n = 22). Given is the average (line) ±SD (filled area), except when single-cell data are shown (green).

(B) Representative kymographs of mitotic cells expressing ΔN-Cdc13 and overexpressing securin-GFP and separase with or without *mad2* deletion (vertical scale bar, 5 μm).

(C and D) Securin-GFP intensity at the time point of reflattening of the securin degradation curve ("securin stabilization") (C) and time between anaphase onset and securin stabilization (D) for the experiment shown in (A). Measurements from single cells (colored) with mean and SD (black lines) are shown.

(E) Representative curve showing checkpoint re-engagement from a cell expressing *mad2+-mCherry* in addition to nondegradable cyclin B and overexpression of securin-GFP and separase. For each time point, the normalized nuclear securin-GFP intensity (green) and the maximal cellular Mad2-mCherry signal in raw camera counts (rcc) (gray) is shown. Δt denotes the time difference between the start of Mad2-mCherry signal increase (red circle) and stabilization of securin-GFP (red cross).

(F) Time difference between start of Mad2-mCherry signal increase and stabilization of securin-GFP (Δt) as shown in (E). Single-cell measurements (red) with mean and SD (black lines) are shown.

See also Figure S3.

checkpoint proteins to kinetochores is typically not observed (Figures 1A, 1C, and S1E). This indicates that chromosome attachments remain stable, providing no possibility for the checkpoint to become engaged. This also indicates that persistent chromosome attachment is the primary mechanism that ensures unperturbed anaphase progression. Slow checkpoint activation (as we describe here) or an inability to activate the checkpoint in anaphase [11] will only become functionally important when the primary mechanism fails. This prompted us to ask how likely this is. Stability of chromosome attachment is thought to be regulated by centromere-localized Aurora B [2, 10, 11]. We therefore wanted to know when with respect to anaphase Aurora B translocates from the centromere to the midspindle and how variable this process is. Surprisingly, there was considerable variability and we found Aurora B on centromeres for up to 2 min after anaphase onset (Figures S4A and S4B), consistent with findings in vertebrate cells [31]. Hence, attachment remains stable despite the presence of Aurora B on centromeres, strengthening previous hints that an additional mechanism supports chromosome attachment stability in anaphase [11].

Like Aurora B translocation [8, 9], this other mechanism seems to require declining CDK1/cyclin B activity, because maintaining high cyclin B levels creates unstable chromosome attachments in anaphase (Figures S1B and S1D). Hence, anaphase is at risk as long as cyclin B levels are still (relatively) high. Checkpoint activation on the other hand is slow even when cyclin B levels are high (Figure 3), making it a suitable mechanism to protect anaphase in such a situation. The cells co-overexpressing securin and separase provide a means to test this idea. In these cells, anaphase occurs very early after the APC/C has become active and Aurora B translocates considerably later with respect to anaphase than in wild-type cells (Figure S4C). Hence, there should be a higher tendency for destabilization of chromosome attachments. Consistently, we sometimes see securin stabilization in these cells, indicating that an error was recognized during anaphase (Figure S4D). This needs to be corroborated by visualizing checkpoint proteins, which technical difficulties have so far rendered impossible for us. Most cells proceeded through anaphase unhindered, which suggests that slow checkpoint activation may be efficient in ensuring anaphase progression while cyclin B levels are still high.

Slow Checkpoint Activation Seems Evolutionary Conserved, Despite the Risks Associated

Since we found slow checkpoint activation kinetics at high cyclin B levels (mimicking the biochemical situation in prometaphase), checkpoint activation may be slow throughout prometaphase. This is surprising because the checkpoint is considered a crucial safety mechanism during that time of mitosis. Yet, this slowness seems evolutionary conserved since the Gerlich group recently reported similarly slow checkpoint activation kinetics during metaphase in human cells [22].

We asked whether such slow checkpoint activation is at all consistent with the timing of mitosis. In an unperturbed *S. pombe* mitosis, it takes on average 5.8 min from entry into mitosis (when chromosomes are initially unattached) to APC/C activation (Figures S4E and S4F). Apparently, this timing is set by checkpoint-independent mechanisms controlling APC/C activity, because deletions of checkpoint genes do not accelerate mitosis (Figure S4G). For the checkpoint to be able to protect chromosomes from missegregating, attachment errors must block APC/C activity in less than 5.8 min.

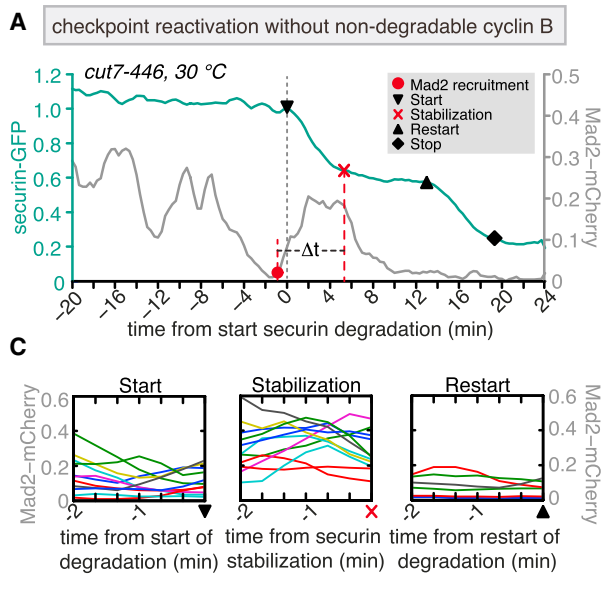

Figure 4. Checkpoint Re-engagement Triggered by Spindle Destabilization during the Metaphase-to-Anaphase Transition

(A) Normalized nuclear securin-GFP intensity (green) and normalized maximal cellular Mad2-mCherry intensity (gray) in a cell progressing through mitosis, expressing the temperature-sensitive kinesin-5 mutant allele *cut7-446* and overexpressing securin-GFP. Red circle, reincrease of Mad2; black head-down triangle, initial start of securin degradation; red cross, stabilization of securin-GFP abundance; black triangle, restart of securin degradation; black diamond, final stabilization of securin-GFP abundance; Δt, time between start of Mad2-mCherry signal increase and stabilization of securin-GFP abundance. A stabilization of securin abundance was observed in 11 cells, which are further analyzed in (B)–(D); only five of these cells showed a restart of securin degradation.

(B) Quantification of the normalized securin-GFP intensities at the different time points of anaphase as shown in (A). Average and SD are shown.

(C) Single-cell measurements of Mad2-mCherry intensities in the 2 min preceding the indicated points defined in (A).

(D) Time difference between start of Mad2-mCherry signal increase and restabilization of securin-GFP (Δt) with respect to the time that elapsed between the start of securin degradation and Mad2 rerecruitment. Shown are single-cell measurements (red circles) and regression line (black).

Our data indicate that it takes on average 4.3 min (in the presence of nondegradable cyclin B; Figure 3F). Hence, there is just enough time for chromosome attachment errors in early mitosis to prevent APC/C activity. In contrast, chromosome attachment errors that occur late in prometaphase may not have enough time to block APC/C activity. This is consistent with observations in human cells [22] and reveals a surprising vulnerability in the checkpoint mechanism. We can envision two possibilities why slow checkpoint activation nevertheless exists and is evolutionary conserved: either there is a biochemical constraint, which makes faster inhibition of the APC/C impossible, or the slowness has been evolutionary conserved because it provides a safety mechanism in anaphase, as our work here suggests.

Experimental Procedures

S. pombe Strains and Growth Conditions

Strains are listed in Table S1. PCR-based gene targeting [32] was used to replace genes by gene fusions at their endogenous loci. For overexpression of securin and separase, we replaced the endogenous promoters by the constitutive *Padh1* [33] and the thiamine-repressible *Pnmt1* promoter [34], respectively—except for Figures 2B and 2C, where separase overexpression was achieved by integration of *Pnmt1-cut1*$^{+}$*-13myc-Tadh1* into the *leu1* locus. For inducible expression of *ΔN-cdc13*, the coding sequence for amino acids 68 to 482 of Cdc13 [16] was cloned into the pDual vector [35] under control of the *Pnmt81* promoter and integrated into the *leu1* locus. Unless stated differently, cells were grown at 30°C in Edinburgh minimal medium (EMM) with the necessary supplements. When applicable, the *nmt* promoter was suppressed by addition of 16 μM thiamine. Protein expression from the *nmt* promoter and its derivatives at 30°C was induced by culturing of the cells for 14–18 hr in EMM without thiamine. Rich medium (YEA) was used for asynchronously growing cells for protein extraction and immunoblotting. Detailed information can be found in the Supplemental Experimental Procedures.

Live-Cell Imaging

Cells were mounted in lectin-coated (35 μg/ml; Sigma L1395) culture dishes (8-well; Ibidi) and preincubated on the microscope stage for 30 min. Live-cell imaging was carried out at 30°C (if not stated otherwise) on a DeltaVision Core system (Applied Precision/GE Healthcare) equipped with a climate chamber (EMBL) using a 60×/1.4 Apo oil objective (Olympus). Images were deconvolved using SoftWorx software. For representative pictures, maximum-intensity projections were used if z stacks were acquired. All intensity measurements were performed using SoftWorx, and data analysis and kymograph assembly were performed using MatLab. Intensity was measured in units of raw camera counts. A detailed description of the imaging conditions and analysis can be found in the Supplemental Experimental Procedures.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.02.005.

Author Contributions

J.K. and S.H. conceived the project and wrote the manuscript; J.K. designed, performed, and analyzed experiments.

Acknowledgments

We thank Tomohiro Matsumoto for antibodies, Yoshinori Watanabe for *S. pombe* strains, Mitsuhiro Yanagida for plasmids, Eva Illgen and Armin Kubis for excellent technical help, members of the Hauf lab for discussions, and Maria Vázquez-Novelle and Mark Petronczki for communicating results prior to publication. This work was supported by the Max Planck Society and the Boehringer Ingelheim Fonds (fellowship to J.K.).

Received: January 9, 2014
Revised: February 2, 2014
Accepted: February 4, 2014
Published: February 27, 2014

References

1. Lampson, M.A., and Cheeseman, I.M. (2011). Sensing centromere tension: Aurora B and the regulation of kinetochore function. Trends Cell Biol. *21*, 133–140.
2. Carmena, M., Wheelock, M., Funabiki, H., and Earnshaw, W.C. (2012). The chromosomal passenger complex (CPC): from easy rider to the godfather of mitosis. Nat. Rev. Mol. Cell Biol. *13*, 789–803.

3. Lara-Gonzalez, P., Westhorpe, F.G., and Taylor, S.S. (2012). The spindle assembly checkpoint. Curr. Biol. *22*, R966–R980.
4. Musacchio, A., and Salmon, E.D. (2007). The spindle-assembly checkpoint in space and time. Nat. Rev. Mol. Cell Biol. *8*, 379–393.
5. Oliveira, R.A., and Nasmyth, K. (2010). Getting through anaphase: splitting the sisters and beyond. Biochem. Soc. Trans. *38*, 1639–1644.
6. Vázquez-Novelle, M.D., Mirchenko, L., Uhlmann, F., and Petronczki, M. (2010). The 'anaphase problem': how to disable the mitotic checkpoint when sisters split. Biochem. Soc. Trans. *38*, 1660–1666.
7. Parry, D.H., Hickson, G.R., and O'Farrell, P.H. (2003). Cyclin B destruction triggers changes in kinetochore behavior essential for successful anaphase. Curr. Biol. *13*, 647–653.
8. Pereira, G., and Schiebel, E. (2003). Separase regulates INCENP-Aurora B anaphase spindle function through Cdc14. Science *302*, 2120–2124.
9. Hümmer, S., and Mayer, T.U. (2009). Cdk1 negatively regulates midzone localization of the mitotic kinesin Mklp2 and the chromosomal passenger complex. Curr. Biol. *19*, 607–612.
10. Mirchenko, L., and Uhlmann, F. (2010). Sli15(INCENP) dephosphorylation prevents mitotic checkpoint reengagement due to loss of tension at anaphase onset. Curr. Biol. *20*, 1396–1401.
11. Vázquez-Novelle, M.D., and Petronczki, M. (2010). Relocation of the chromosomal passenger complex prevents mitotic checkpoint engagement at anaphase. Curr. Biol. *20*, 1402–1407.
12. Oliveira, R.A., Hamilton, R.S., Pauli, A., Davis, I., and Nasmyth, K. (2010). Cohesin cleavage and Cdk inhibition trigger formation of daughter nuclei. Nat. Cell Biol. *12*, 185–192.
13. Tinker-Kulberg, R.L., and Morgan, D.O. (1999). Pds1 and Esp1 control both anaphase and mitotic exit in normal cells and after DNA damage. Genes Dev. *13*, 1936–1949.
14. Palframan, W.J., Meehl, J.B., Jaspersen, S.L., Winey, M., and Murray, A.W. (2006). Anaphase inactivation of the spindle checkpoint. Science *313*, 680–684.
15. Wolf, F., Wandke, C., Isenberg, N., and Geley, S. (2006). Dose-dependent effects of stable cyclin B1 on progression through mitosis in human cells. EMBO J. *25*, 2802–2813.
16. Yamano, H., Gannon, J., and Hunt, T. (1996). The role of proteolysis in cell cycle progression in Schizosaccharomyces pombe. EMBO J. *15*, 5268–5279.
17. Dischinger, S., Krapp, A., Xie, L., Paulson, J.R., and Simanis, V. (2008). Chemical genetic analysis of the regulatory role of Cdc2p in the S. pombe septation initiation network. J. Cell Sci. *121*, 843–853.
18. Mulvihill, D.P., Petersen, J., Ohkura, H., Glover, D.M., and Hagan, I.M. (1999). Plo1 kinase recruitment to the spindle pole body and its role in cell division in Schizosaccharomyces pombe. Mol. Biol. Cell *10*, 2771–2785.
19. Biggins, S., and Murray, A.W. (2001). The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. Genes Dev. *15*, 3118–3129.
20. Windecker, H., Langegger, M., Heinrich, S., and Hauf, S. (2009). Bub1 and Bub3 promote the conversion from monopolar to bipolar chromosome attachment independently of shugoshin. EMBO Rep. *10*, 1022–1028.
21. Hagan, I., and Yanagida, M. (1990). Novel potential mitotic motor protein encoded by the fission yeast cut7+ gene. Nature *347*, 563–566.
22. Dick, A.E., and Gerlich, D.W. (2013). Kinetic framework of spindle assembly checkpoint signalling. Nat. Cell Biol. *15*, 1370–1377.
23. Hagting, A., Den Elzen, N., Vodermaier, H.C., Waizenegger, I.C., Peters, J.-M., and Pines, J. (2002). Human securin proteolysis is controlled by the spindle checkpoint and reveals when the APC/C switches from activation by Cdc20 to Cdh1. J. Cell Biol. *157*, 1125–1137.
24. Qi, W., and Yu, H. (2007). KEN-box-dependent degradation of the Bub1 spindle checkpoint kinase by the anaphase-promoting complex/cyclosome. J. Biol. Chem. *282*, 3672–3679.
25. King, E.M., van der Sar, S.J., and Hardwick, K.G. (2007). Mad3 KEN boxes mediate both Cdc20 and Mad3 turnover, and are critical for the spindle checkpoint. PLoS ONE *2*, e342.
26. Choi, E., Choe, H., Min, J., Choi, J.Y., Kim, J., and Lee, H. (2009). BubR1 acetylation at prometaphase is required for modulating APC/C activity and timing of mitosis. EMBO J. *28*, 2077–2089.
27. Chung, E., and Chen, R.H. (2003). Phosphorylation of Cdc20 is required for its inhibition by the spindle checkpoint. Nat. Cell Biol. *5*, 748–753.
28. D'Angiolella, V., Mari, C., Nocera, D., Rametti, L., and Grieco, D. (2003). The spindle checkpoint requires cyclin-dependent kinase activity. Genes Dev. *17*, 2520–2525.
29. Morin, V., Prieto, S., Melines, S., Hem, S., Rossignol, M., Lorca, T., Espeut, J., Morin, N., and Abrieu, A. (2012). CDK-dependent potentiation of MPS1 kinase activity is essential to the mitotic checkpoint. Curr. Biol. *22*, 289–295.
30. Yamaguchi, S., Decottignies, A., and Nurse, P. (2003). Function of Cdc2p-dependent Bub1p phosphorylation and Bub1p kinase activity in the mitotic and meiotic spindle checkpoint. EMBO J. *22*, 1075–1087.
31. Murata-Hori, M., Tatsuka, M., and Wang, Y.L. (2002). Probing the dynamics and functions of aurora B kinase in living cells during mitosis and cytokinesis. Mol. Biol. Cell *13*, 1099–1108.
32. Bähler, J., Wu, J.Q., Longtine, M.S., Shah, N.G., McKenzie, A., 3rd, Steever, A.B., Wach, A., Philippsen, P., and Pringle, J.R. (1998). Heterologous modules for efficient and versatile PCR-based gene targeting in Schizosaccharomyces pombe. Yeast *14*, 943–951.
33. McLeod, M., Stein, M., and Beach, D. (1987). The product of the mei3+ gene, expressed under control of the mating-type locus, induces meiosis and sporulation in fission yeast. EMBO J. *6*, 729–736.
34. Basi, G., Schmid, E., and Maundrell, K. (1993). TATA box mutations in the Schizosaccharomyces pombe nmt1 promoter affect transcription efficiency but not the transcription start point or thiamine repressibility. Gene *123*, 131–136.
35. Matsuyama, A., Shirai, A., Yashiroda, Y., Kamata, A., Horinouchi, S., and Yoshida, M. (2004). pDUAL, a multipurpose, multicopy vector capable of chromosomal integration in fission yeast. Yeast *21*, 1289–1305.